Lecture 32
5/5/04

Exam 4-153 7:30

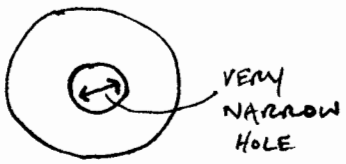
Proteosome structure- 2 to 4 seven membered rings stacked depending on whether it is in a prokaryote (2) or a eucaryote (4)

Euk- usually 4 rings, additional complexity (alpha, beta, beta, alpha)
Pro- usually 2 rings, example ClpP (well studied)

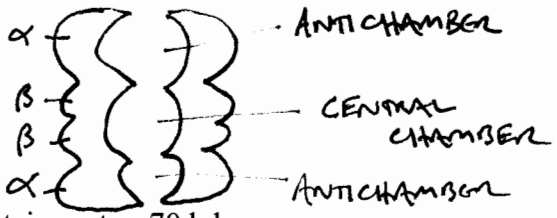
Thermoplasma proteosome – 4 stacked rings each with 7 subunits
(alpha beta beta alpha) – sandwich
alpha and beta subunits are structurally homologous, but there are some differences
alpha is inactive, beta is active (and is synthesized in a pro-protein, inactive form)

The hole through the proteosome is way too small to fit a folded, globular protein (w/out some large conformational change) – need to unfold protein to get it into the proteosome

Top view



Side view (cross section)



Central chamber could hold unfolded protein up to ~70 kda

How does a protein unfold and translocate to get into the chamber in which it is then degraded?
No energy is required for the proteolysis (just like no energy is required for peptide bond formation)

ClpP-> 2 stacked rings, simplest model- all subunits are the same

Human proteosome (see p. 13 handout 4a) – 26S proteosome (4 stacked rings w/ lids on each end) The subunits of the proteosome are also isozymes.
There are different kinds of lids

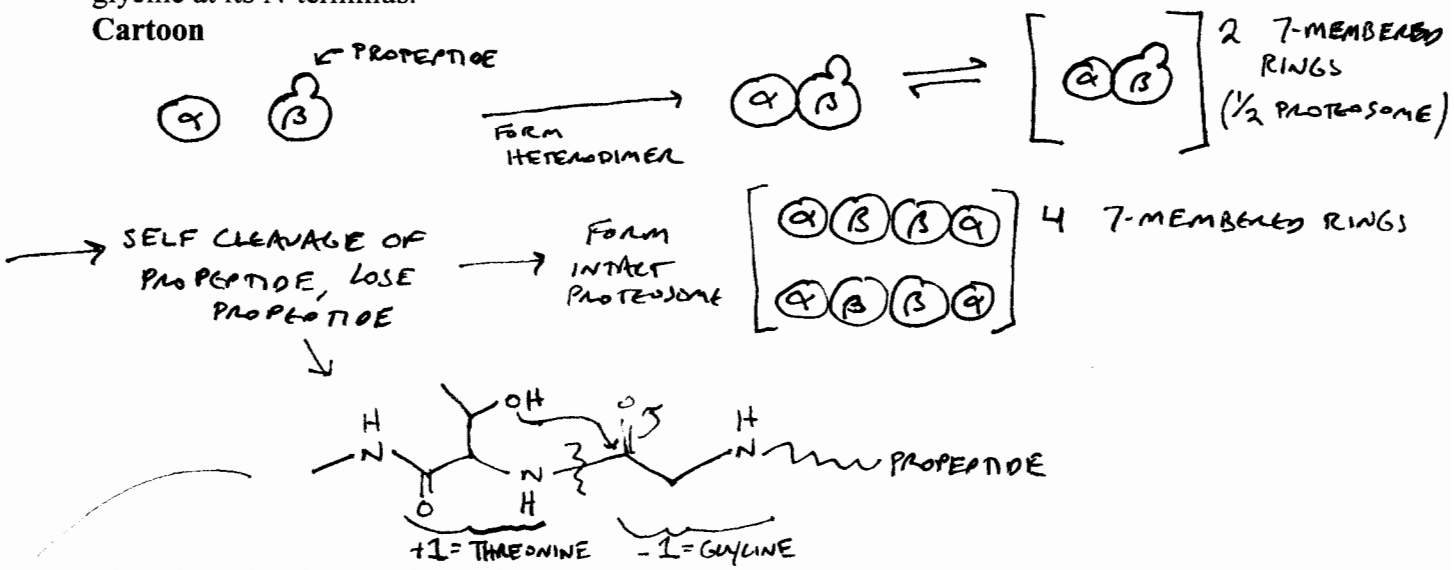
Processing mechanism (euk) See page 13 handout 4a
Self-processing of beta from pro-protein to active form and the mechanism of self processing in conserved from archae to euk
Archae
Alpha and beta-pro-protein interact and form heterodimer-> then form one half of a proteosome (two stacked 7 membered rings)-> self cleavage of pro-peptide and loss of propeptide-results in formation of the intact proteosome

Chemical processing mechanism

Threonine is in the +1 position (after bond to be cleaved), glycine is in the –1 position (key is glycine's flexibility)

T acts as the nucleophile in peptide bond hydrolysis to the adjacent glycine. It forms a tetrahedral intermediate, that then collapses concomitant with cleavage of the peptide bond and liberates the N-terminus of the protein (the amino group of T). This process leaves an acyl enzyme with an ester linkage. The ester linkage is then hydrolyzed again through tetrahedral intermediate to release active beta subunit and propeptide with glycine at its N-terminus.

Cartoon

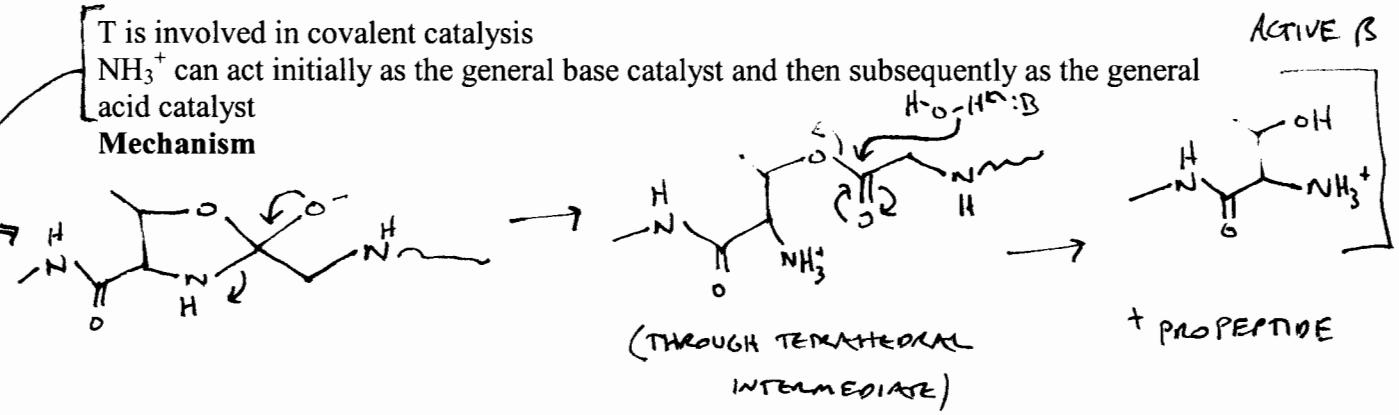


The released active beta is the liberated catalyst

T is involved in covalent catalysis

NH_3^+ can act initially as the general base catalyst and then subsequently as the general acid catalyst

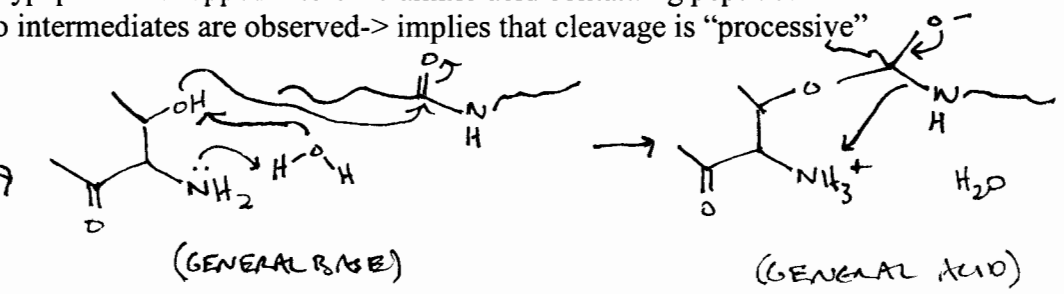
Mechanism



In the bacterial proteosome (ClpP) -> the mechanism is completely analogous to chymotrypsin mechanism, with S,H,D in the active site.

Proteosomes don't degrade the substrate protein down to single amino acids, the polypeptide is chopped into 6-10 amino acid containing peptides

No intermediates are observed-> implies that cleavage is "processive"



Questions: specificity? How does it cleave down to only peptides of between 6-10 amino acids? Does it start chopping off pieces from one end, or does it chop somewhere in the middle, and then again in the middle of that…active area of research

p. 13 of handout 4a
In yeast-> 7 alpha subunits (same structure, different sequences that is isozymes)
It has 7 beta subunits, but only 3 are active (analogous to different proteases- one is like trypsin, one like chymotrypsin, and one recognizes glutamate side chains…)
Reconstitution of the yeast proteosome has been not trivial because of the isozyme problem!

Tools to study proteolysis- > kill activity may allow you to examine which proteins are targeted for degradation.
Site directed mutagenesis of active site
Change T or S to an A. One would then have to replace the normal gene in the organism with the mutant gene. This can be readily done in both bacteria and yeast (even by a chemist).
A second way to destroy the catalytic activity is by use of small molecule inhibitors like isopropyl fluorophosphates(general) or lactocystin(works uniquely on the eucaryotic proteosomes and not bacteria)
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III. Accessory Proteins (regulatory complex- RC)

All use energy to denature and translocate the proteins that have been targeted for degradation.
All have an AAA+ type ATPase domains-(to unfold protein to be degraded and to translocate the unfolded protein into crypt)

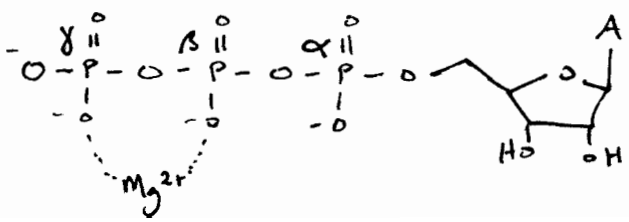
AAA^+ = **A**TPase **A**ssociated with diverse cellular **A**ctivities

Very broad functions in cells (see page 14 handout 4a)

Structure of AAA+ ATPase – general overview (230-250 amino acids)

ATP binding motifs
Walker A (GXXGXGKT)
Walker B (DEID(X)…R)

In cell- 1-3mM ATP, ~2-10 mM Mg^{2+}, given the Kd, 100% of ATP will be complexed with Mg^{2+}
ATP and Mg^{2+}



Mg^{2+} can be bound to the gamma and beta phosphates, or beta, alpha, or alpha, beta gamma, etc…The coordination to Mg^{2+} can also change during catalysis requiring a flexible active site.

Glycines in the Walker A motif provide flexibility

Mg^{2+} is oxophilic, and is octahedrally coordinated.
In addition to phosphates of ATP, it uses H_2O ligands, and D and E, sometimes N, Q (Walker B)
Structure- 6 subunits- hexameric ring structure
Homo-oligomeric (bacteria) and hetero-oligomeric (euk)

The active site is located at the interface of 2 subunits

R in the walker B motif is conserved to neutralize some – charge on phosphates of ATP

Specifity-> Is the AAA^+ type ATPase involved in protein recognition of the protein to be degraded? Yes The substrate can bind directly to the AAA+ domain itself; can bind to a domain attached to the AAA+ domain; or can bind to an adaptor protein that can bind to a domain of the AAA+ domain.